In your eyes…
What eye wrinkles in horses tell us about their emotional state

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Wrinkles above the eye ball are common in domestic horses but may differ in number and shape both between and within individuals. They are caused by contraction of the inner eye brow raiser, and some people working with horses call them "worry wrinkles", considering them to reflect emotional states. However, as yet no study has formally investigated the relationship between eye wrinkles and emotional state in horses.

The aim of the present study was to induce states of different emotional valence and to assess whether positive emotional states would reduce the expression of eye wrinkles while negative emotional states would increase it. Sixteen horses were confronted in a balanced order with two presumably positively and two negatively valenced situations each. Positive situations included anticipation of a food reward (FA) and petting (P), negative situations included food competition (FC) and waving a plastic bag (PB). Each situation lasted for 60s (TRT) and was preceded by a 60s control phase (CON). Throughout CON and TRT pictures of the eyes were taken, and for each horse four pictures per situation (FA, P, FC, PB) and phase (CON and TRT) were randomly selected (n = 512) and scored in random order and blind to treatment for six outcome variables: overall impression (qualitative), number, angle and markedness of eye wrinkles, presence of eye white, and shape of eye lid.

Data were analysed separately for the right and left eye using linear mixed effects models (angle, number), generalised linear mixed models (eye white, markedness), and ordered logistic regression (qualitative, shape of eye lid), with "situation" (FA, P, FC, PB), "phase" (CON, TRT) and their two-way interaction as fixed effects.

Expression of eye wrinkles did not vary consistently across "situation" and "phase". Independent of phase, eye white appeared less frequently during P than during FA ($z=-3.15$, $p=0.009$), FC ($z=-2.94$, $p=0.02$), and PB ($z=4.17$, $p<0.001$) in the left eye and during PB ($z=4.10$, $p\ 0.001$) in the right eye. Similarly, wrinkles were less marked during P compared to the other situations in the left eye (FA: $z=3.15$, $p=0.009$; FC: $z=-2.94$, $p=0.017$; PB: $z=4.17$, $p<0.001$) and compared to PB in the right eye ($z=4.10$, $p=0.001$), while no differences between situations occurred in number of wrinkles, overall impression and shape of eye lid for both eyes. Consistent with our hypothesis, P induced relaxation of the underlying muscle in the right eye resulting in a wider angle compared to its control phase (interaction situation*phase: $F_{3,10}=3.71$, $p=0.055$; post-hoc comparison: $z=-3.57$, $p=0.009$), while FC induced muscle contraction, resulting in a sharper angle in the left eye (interaction situation*phase: $F_{3,11}=6.57$, $p=0.011$; $z=3.73$, $p=0.005$).

We conclude that emotional valence may affect characteristics of eye winkle expression in horses which might therefore be a promising indicator of horses' emotional states, but further research is needed to validate the relevant outcome variables.

Keywords: eye wrinkles, emotional valence, positive and negative emotions, welfare assessment

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